PROJECT NUMBER: 6908
PROJECT TITLE: Smoke Condensate Studies
PROJECT LEADER: A. H. Warfield
PERIOD COVERED: November, 1989

I. UNEXTRACTED NICOTINE STUDIES (with W. Hempfling)

A. Objective: To determine whether there is a correlation between unextracted nicotine (UN) in filler and the delivery of NNK in the corresponding MS smoke.

B. Results: Supercritical CO_2 extracted DBC-Bright, DBC-Burley, and MT-Oriental fillers were obtained from Physical Research Division, and values for TARN,[1] EN,[2] Nic-X[3] and Nic-Y[4] were determined using previously described methods. TARN for all the fillers was in the range 1200-1900 ppm, and EN varied from 40% of TARN (Or) to 61% of TARN (Br). Digestion of the hot water extracts gave Nic-X values of 190 ppm (Or) to 389 ppm (Br). Nic-Y values were then calculated by subtracting the total of EN + Nic-X from the TARN values. When the marcs from the water extractions were treated with hot alkali, direct measurements of Nic-Y were obtained. Unexpectedly, the direct Nic-Y values were only 42%, 35%, and 14% of the calculated Nic-Y values, respectively.

A possible explanation for these discrepancies is suggested by the data obtained for nornicotine (NN) on the same samples. TARNN[5] values for the three fillers ranged from 1214 ppm (Or) to 3233 ppm (Bu). ENN[6] varied from 62% of TARNN (Or) to 100% of TARNN (Bu). Amounts of NN-X[7] that were found after digestion of the hot water extracts in hot alkali were not statistically significant. All of the NN was extracted from burley with hot water, but 355 ppm of NN was still released from the hot water burley marc by hot alkali digestion. The sum of Nic-Y and NN-Y[8] nearly equaled the amount of Nic-Y expected (624 ppm). However, no NN-Y was found in the oriental marc, although ~400 ppm was expected.

C. Plans: Repeat the extractions of SCFE fillers and submit for analysis to confirm the results obtained above. Compare nicotine content in Bu-21 green samples as determined by our method with those found by pyrolysis (ARD).

D. References:

Lambert, E. A. Notebook No. 8852, p. 59.
Keene, C. K. Notebook No. 8754, p. 190.

E. Footnote:

[1]TARN = total alkali-releasable nicotine. [2]EN = nicotine extractable by hot water. [3]Nic-X = hot-water-soluble alkali-releasable nicotine. [4]Nic-Y = hot-water-insoluble alkali-releasable nicotine. [5]TARNN = total alkali-releasable nornicotine. [6]ENN = nornicotine extractable by hot water. [7]NN-X = hot-water-soluble

alkali-releasable nornicotine. [8]NN-Y = hot-water-insoluble alkali-releasable nornicotine.

II. ORIENTAL INHIBITOR STUDIES

A. **Objective**: To determine whether the causative agent(s) responsible for the reduced levels of MS TSNA observed for Or tobacco can be removed with organic solvents or supercritical CO_2 and applied to other fillers as a means of decreasing the MS TSNA levels delivered by these fillers.

B. **Results**: The SCF CO_2 extract of blended Or filler, prepared at the facilities of Supercritical Processing, Inc., has been examined by TLC and an initial fractionation into three polarity categories has been performed. A major component was shown to be solanesol, which afforded no reduction in MS TSNA in earlier tests. Chemical classification of the subfractions is in progress, and additional material is being generated for further studies.

C. **Plans**: The fractions will be tested for inhibition of TSNA pyrosynthesis.

D. **Reference**:

Haut, S. A. Notebook No. 8891, p. 11.

III. TSNA REMOVAL STUDIES

A. **Objective**: To prepare a blend of fillers reduced in endogenous TSNA and minor alkaloid TSNA precursors, which can be used to make a laboratory cigarette with reduced MS TSNA delivery relative to that of a 1987 full-flavored, blended cigarette.

B. **Results**: DBC Bright and DBC Burley fillers were previously extracted with 95% ethanol using the cation exchange process to remove alkaloids and TSNA. Attempts were made this month to reapply the concentrated solids, minus the alkaloids and TSNA, onto the respective fillers in order to prepare the alkaloid and TSNA-depleted fillers as blend components. The initial attempt to spray the extract concentrates resulted in only a 30% recovery of solids. The second attempt gave better results, but the recovery of solids was still only ~75%.

C. **Plans**: Analyze the above fillers for alkaloids and TSNA, blend the burley and bright fillers with oriental, and smoke for MS TSNA.

D. **Reference**:

Tickle, M. H. Notebook No. 8716, p. 200.

IV. TSNA PRECURSORS

A. **Objective**: To determine the precursors of MS TSNA.

B. **Results**: Fresh samples of Br and Bu RLs were obtained from R. Izac (Project 6912) for the purpose of initiating an RL aging study. Zero-time filler TSNA data were obtained. Samples that have been aged five weeks at 45°C have also been analyzed for filler TSNA, along with corresponding sampled stored at -30°C. The data obtained to date are inconclusive.

C. **Plans**: Further data on the RL aging study will be obtained. Plans are being made for a study of possible ways to remove UN from filler.

D. **Reference**:

Haut, S. A. Notebook No. 8891, p. 11.

V. MISCELLANEOUS AND SUPPORT STUDIES

A. **Objective**: To conduct studies of the TSNA content of filler and/or MS smoke or carry out other activities as necessary to support other PM programs.

B. **Results**: SAVA smokings of test cigarettes were conducted to determine whether various cigarette papers had any effect on either MS or SS nitrosamine levels. No differences due to cigarette paper were found.

C. **Reference**:

Morgan, W. R. Notebook No. 8905, pp. 1-2.